

TRANSMITTAL LETTER TO THE UNITED STATES  
DESIGNATED/ELECTED OFFICE (DO/EO/US)  
CONCERNING A FILING UNDER 35 U.S.C. 371

2727-99J

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR 1.5)

09/463209

INTERNATIONAL APPLICATION NO.  
PCT/EP98/04510INTERNATIONAL FILING DATE  
21 July 1998PRIORITY DATE CLAIMED  
21 July 1997

## TITLE OF INVENTION

NUCLEIC ACID MOLECULE, TEST KIT AND USE

## APPLICANT(S) FOR DO/EO/US

Kornelia Berghof, Alexander Gasch, Freimut Wilborn and Arndt Rolfs

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
  - a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
  - b. ☐ has been transmitted by the International Bureau.
  - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☐ A copy of the International Search Report (PCT/ISA/210).
8. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
  - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau)
  - b. ☐ have been transmitted by the International Bureau.
  - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
  - d. ☐ have not been made and will not be made.
9. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
10. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
11. ☐ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

## Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☐ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ Certificate of Mailing by Express Mail
20. ☒ Other items or information:

Amendments to Claims Under Article 34 (in German)

Declaration (unsigned)

Cover Sheet of WIPO Publication

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR 1.5)		INTERNATIONAL APPLICATION NO.		ATTORNEY'S DOCKET NUMBER	
09/463209		PCT/EP98/04510		2727-99J	
21. The following fees are submitted: <b>BASIC NATIONAL FEE ( 37 CFR 1.492 (a) (1) - (5) ) :</b> <input type="checkbox"/> Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO ..... \$970.00 <input checked="" type="checkbox"/> International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO ..... \$840.00 <input type="checkbox"/> International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO ..... \$690.00 <input type="checkbox"/> International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) ..... \$670.00 <input type="checkbox"/> International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) ..... \$96.00 <b>ENTER APPROPRIATE BASIC FEE AMOUNT =</b>				<b>CALCULATIONS PTO USE ONLY</b>  <div style="border: 1px solid black; height: 100px; width: 100%;"></div>	
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).				\$0.00	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	- 20 =	0	x \$18.00	\$0.00	
Independent claims	- 3 =	0	x \$78.00	\$0.00	
Multiple Dependent Claims (check if applicable).				\$0.00	
<b>TOTAL OF ABOVE CALCULATIONS</b>				<b>= \$840.00</b>	
Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) (check if applicable).				\$0.00	
<b>SUBTOTAL</b>				<b>= \$840.00</b>	
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492 (f)).				\$0.00	
<b>TOTAL NATIONAL FEE</b>				<b>= \$840.00</b>	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) (check if applicable).				\$0.00	
<b>TOTAL FEES ENCLOSED</b>				<b>= \$840.00</b>	
				Amount to be:	\$
				refunded	\$
				charged	\$
<input type="checkbox"/> A check in the amount of _____ to cover the above fees is enclosed.					
<input checked="" type="checkbox"/> Please charge my Deposit Account No. <b>501145</b> in the amount of <b>\$840.00</b> to cover the above fees A duplicate copy of this sheet is enclosed. <b>(Order No. 2727-99J)</b>					
<input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. <b>501145</b> . A duplicate copy of this sheet is enclosed. <b>(Order No. 2727-99J)</b>					
<b>NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.</b>					
SEND ALL CORRESPONDENCE TO:					
<b>Ronald R. Santucci</b> Pitney, Hardin, Kipp & Szuch, LLP 711 Third Avenue, 20th Floor New York, New York 10017  (212)687-6000			<div style="text-align: center;">                       SIGNATURE                 </div> <div style="text-align: center;"> <b>Ronald R. Santucci</b>                      NAME   <b>28,988</b>                      REGISTRATION NUMBER   <b>January 20, 2000</b>                      DATE                 </div>		

2727-99J

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Kornelia Berghof, Alexander Gasch, Charles  
Mason-Brown, Freimut Wilborn, Arndt Rolfs

Serial No.: 09/463,209  
International Appln. No.: PCT/EP98/04510  
International Filing Date: July 21, 1998  
Priority Date Claimed: July 21, 1997  
For: NUCLEIC ACID MOLECULE, KIT AND USE

PRELIMINARY AMENDMENT

Box PCT  
Commissioner for Patents  
Washington, D.C. 20231  
Attn: DO/EO/US

S I R:

Preliminary to examination of the above-identified  
application kindly amend the application as follows:

In the Claims:

In claim 9, lines 1, 3-4, 6-7, 9-10, 12-13, and 15, each  
time kindly delete "any one of the preceding claims" and  
substitute therefor --claim 1--;

In claim 11, line 1, kindly delete "any one of the  
preceding claims" and substitute therefor --claim 1--;

In claim 12, line 1, kindly delete "any one of the  
preceding claims" and substitute therefor --claim 1--;

In claim 14, line 1, kindly delete "or claim 13";

In claim 15, lines 1-2, kindly delete "any one of the  
preceding claims" and substitute therefor --claim 1--;

In claim 18, line 1, kindly delete "any one of claims 15  
to 17" and substitute therefor --claim 15--;

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Express Mail No: EK052279325US

In claim 20, line 1, kindly delete "any one of claims 15 to 19" and substitute therefor --claim 15--;

In claim 30, lines 5, 7-8, 10-11 and 13, each time kindly delete "any one of claims 22 to 29" and substitute therefor --claim 22--;

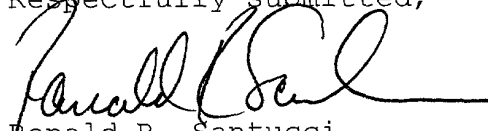
In claim 32, lines 1-2, kindly delete "any one of claims 22 to 30" and substitute therefor --claim 22--;

In claim 33, lines 1-2, kindly delete "any one of claims 22 to 32" and substitute therefor --claim 22--.

REMARKS

The specification of the above-identified application has been amended to remove all multiple dependencies. (Please note that the claims being amended are those previously amended according to Article 34 of Chapter II PCT.) No new matter has been added. Accordingly, an early examination of the application is respectfully requested.

Respectfully submitted,



Ronald R. Santucci  
Registration No. 28,988

Pitney, Hardin, Kipp & Szuch, LLP  
711 Third Avenue, 20th Floor  
New York, New York 10017  
212-687-6000

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(f) AND 1.27 (c)) - SMALL BUSINESS CONCERN					Docket No. 2737-293	
Serial No. 09/463,209	Filing Date	Patent No.		Issue Date		
Applicant: Kornelia Berghof, Alexander Gaeck, Charles Mason-Brown, Freimut Wilborn, Arndt Ralfs						
Invention: NUCLEIC ACID MOLECULE, TEST KIT AND USE						
<p>I hereby declare that I am:</p> <p><input type="checkbox"/> the owner of the small business concern identified below.</p> <p><input checked="" type="checkbox"/> an official of the small business concern empowered to act on behalf of the concern identified below.</p> <p>NAME OF CONCERN: <u>Bloticon Gesellschaft fuer Biotechnologische Entwicklung und Consulting mbH</u></p> <p>ADDRESS OF CONCERN: <u>Tegeler Weg 33, D-10589, Berlin, Germany</u></p> <p>I hereby declare that the above-identified small business concern qualifies as a small business concern as defined in 13 CFR 121.3-18, and reproduced in 37 CFR 1.9(d), for purposes of paying reduced fees under Section 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.</p> <p>I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the above identified invention described in:</p> <p><input type="checkbox"/> the specification filed herewith with title as listed above.</p> <p><input checked="" type="checkbox"/> the application identified above.</p> <p><input type="checkbox"/> the patent identified above.</p> <p>If the rights held by the above-identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed on the next page and no rights to the invention are held by any person, other than the inventor, who could not qualify as an independent inventor under 37 CFR 1.9(c) or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).</p>						

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Postscript

Patent and Trademark Office-U.S. DEPARTMENT OF COMMERCE

Each person, concern or organization to which I have assigned, granted, conveyed, or licensed or am under an obligation under contract or law to assign, grant, convey, or license any rights in the invention is listed below.

- ☒ no such person, concern or organization exists.  
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Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING:

K. Berghof

TITLE OF PERSON SIGNING

Director

OTHER THAN OWNER:

ADDRESS OF PERSON SIGNING:

Tegler Weg 33, D-10589,  
Berlin, Germany

SIGNATURE:

(K. Berghof)

DATE:

16.3.2000

Patent and Trademark Office U.S. DEPARTMENT OF COMMERCE

09/463209

**Nucleic acid molecule, kit and use****General background of the invention**

A bacterium of great clinical relevance is the gram-positive bacterium *Staphylococcus aureus*. It is one of the most frequent causes of nosocomial infections (infections transmitted in hospital), the spread of which is difficult to control because of the occurrence of various forms of antibiotic resistance (for example, resistance to methicillin and vancomycin). *Staphylococcus aureus* is also one of the most frequent causes of food poisoning, which is generally caused by enterotoxins. The frequent occurrence of *Staphylococcus aureus* in foodstuff products therefore requires a regular investigation of potentially contaminated products. Conventional microbiological methods for the detection of *Staphylococcus aureus* are very time-consuming (at least 4 days). There is therefore a great need for alternative methods, by means of which the presence of *Staphylococcus aureus* can be diagnosed rapidly and reliably in the course of the production process (from the raw materials to the finished product).

A number of new methods for routine use in the detection of microorganisms have been developed in recent years. These include immunological methods based on the use of polyvalent or monoclonal antibodies, and methods in which nucleic acid probes are used for detection by means of hybridisation to organism-specific nucleic acids. Further methods described are those based on amplification of a specific nucleic acid, with or without a subsequent confirmation reaction by nucleic acid hybridisation.

Methods used for the amplification of nucleic acids are, for example, polymerase chain reaction (PCR) [US Patents 4,683,195; 4,683,202; and 4,965,188], ligase chain reaction [WO Publication 89/09835], "self-sustained sequence replication" [EP 329 822], the "transcription based amplification system" [EP 310 229] and the Q $\beta$  RNA-replicase system [US Patent 4,957,858].

The nucleic-acid-based methods mentioned are so sensitive that, unlike in conventional microbiological methods, the tedious process of increasing the quantity of the microorganism to be detected from the sample to be investigated is unnecessary. A test for the presence or absence of the microorganism in question is therefore generally concluded within one working day when using the nucleic-acid-based methods mentioned. This constitutes a considerable time saving, in particular when several days to several weeks are needed for detection by conventional methods.

A number of rapid tests have recently been developed by means of which the presence of *Staphylococcus aureus* can be significantly shortened. These include coagulase tests, various agglutination tests, TNase tests (enzyme activity, monoclonal antibodies), DNA hybridisation tests and PCR tests [for an overview, see BRAKSTAD and MAELAND, (1995), APMIS 103, 209-218]. PCR tests are superior to the other methods in terms of speed and specificity. The PCR tests described hitherto for the specific detection of the *Staphylococcus aureus* species are based on the gene for thermostable nuclease [*nuc*, Brakstad et al., (1992), J.



Clin. Microbiol. 30, 1654-1660] or on a regulator gene essential for methicillin resistance (*femA*), which is specific for *Staphylococcus aureus* [Ünal et al., (1992), J. Clin. Microbiol. 30, 1685-1691; Vannuffel et al., (1995), J. Clin. Microbiol., 33, 2864-2867]. Using those PCR systems, it has been possible to detect all the investigated species of *Staphylococcus aureus*. It is unclear, however, whether it is also possible to detect coagulase-negative strains of the species *Staphylococcus aureus* using those PCR systems.

The aim of the present invention was to establish a PCR system, the use of which as primers and/or probes ensures as complete detection as possible of all the representatives of the species *Staphylococcus aureus*.

Depending upon the size of the group of microorganisms to be detected and on the evolutionary relationship of microorganisms to be delimited (not to be detected), detection based on differential DNA sequences requires very comprehensive preliminary work in order to find suitable DNA sequences having the desired specificity in each case. The invention described herein relates to DNA sequences by means of which the rapid detection of bacteria of the genus *Staphylococcus*, especially of *Staphylococcus aureus*, is possible.

### Description of the invention

To detect specific microorganisms by means of nucleic acid hybridisation or amplification, organism-specific oligonucleotides are used. **Organism-specific oligonucleotides**

are nucleic acids, from 10 to 250 bases long (preferably from 15 to 30 bases long), the base sequence of which is characteristic for a specific microorganism or a group of microorganisms. Hybridisation to DNA or amplification of DNA using those organism-specific oligonucleotides (for example as primers or probes) in the above-mentioned methods can be effected, under suitable reaction conditions, only when the DNA of the microorganisms to be detected is present.

Prokaryotic ribosomes generally contain three distinct nucleic acid components, generally known as 5S, 16S and 23S rRNA (ribosomal ribonucleic acid). The genetic information for those ribonucleic acids (rDNA) is typically arranged in the genome in the form of tandems. The typical organisation of such a unit is 16S-23S-5S, the three genes being separated from one another by short hypervariable intergenic regions. A plurality of units are present in the genome, it being possible for the number of repeating units to vary in different bacteria. The high degree of conservation of the DNA sequence in the region of the 16S rDNA, the 23S rDNA and the 5S rDNA throughout the entire bacterial kingdom makes it possible to design **non-specific oligonucleotides** even without precise knowledge of the DNA sequences of the microorganisms to be investigated. Such non-specific oligonucleotides are characteristic for a relatively large, generally phylogenetically related group of microorganisms. By using those non-specific oligonucleotides, the person skilled in the art will be able, for example following appropriate preliminary tests by DNA amplification by means of PCR, to isolate rDNA fragments, for example of the 23S/5S intergenic region, of any desired

microorganism. By DNA sequencing, it is then possible to determine the sequence of the hypervariable intergenic regions of the microorganism in question.

DNA sequencing of the 23S/5S intergenic region of as large as possible a number of bacteria **to be detected** (for example of various *Staphylococcus* species) on the one hand and a subsequent comparison of those DNA sequences on the other hand make it possible to locate DNA regions that are not modified or are modified only insignificantly in the group under investigation (e.g. all *Staphylococcus* species).

DNA sequencing of the 23S/5S intergenic region of as large as possible a number of bacteria **that are not to be detected** (for example all bacteria not belonging to the genus *Staphylococcus*) on the one hand and a subsequent comparison of those DNA sequences with the sequence of the bacteria to be detected (e.g. various *Staphylococcus* species) on the other hand make it possible to locate DNA sequences that are characteristic for the bacteria to be detected (e.g. all *Staphylococcus* species). From those DNA sequences it is possible in turn to derive oligonucleotides that can be used as primers and/or probes in nucleic-acid-based methods, with the aim of detecting specifically the group of bacteria in question (e.g. all species of the genus *Staphylococcus*).

The organism-specific oligonucleotides described in the present invention for the detection of bacteria of the genus *Staphylococcus*, especially bacteria of the species *Staphylococcus aureus*, correspond to the 23S/5S intergenic

region and the directly adjacent region of the 23S rDNA. The DNA sequence in that region was determined for a large number of bacteria. Following precise sequence comparisons, organism-specific nucleic acid sequences were determined, from which it is possible to derive primers and/or probes for use in a species- or genus-specific detection method.

The problem underlying the invention is solved according to an embodiment by a nucleic acid molecule that hybridises selectively to RNA or DNA of a group of bacteria of the genus *Staphylococcus*, characterised in that it contains at least 10 successive nucleotides of the region from -113 to +58 relative to the 3'-end of the 23S rDNA of a *Staphylococcus* isolate or their complementary nucleotides. The purpose of the selective hybridisation according to the invention is to detect the mentioned group of bacteria of the genus *Staphylococcus*.

A further embodiment of the invention relates to a nucleic acid molecule that hybridises selectively to RNA or DNA of a group of bacteria of the genus *Staphylococcus*, characterised in that it contains at least 10 successive nucleotides of the region from -113 to +58 relative to the 3'-end of the 23S rDNA of *Staphylococcus aureus* (ATCC 6538) or their complementary nucleotides.

A further embodiment of the invention relates to a nucleic acid molecule that hybridises selectively to RNA or DNA of a group of bacteria of the genus *Staphylococcus*, characterised in that it contains at least 10 successive nucleotides of the region from

- (i) nucleotide position 54 to 83 of SEQ ID NO 1, or
- (ii) nucleotide position 100 to 166 of SEQ ID NO 1, or
- (iii) the sequences complementary to (i) or (ii).

A further embodiment of the invention relates to a nucleic acid molecule for the detection of the presence or absence of bacteria belonging to a group of bacteria of the genus *Staphylococcus*, characterised in that it makes it possible by means of nucleic acid hybridisation and/or nucleic acid amplification methods under suitable reaction conditions to distinguish between bacteria to be detected and bacteria that are not to be detected and that the distinction is possible or is facilitated by a differing nucleic acid sequence at at least one base position in the region of SEQ ID NO: 1, or of its complementary sequence, in the genomic DNA and/or RNA of bacteria to be detected and bacteria that are not to be detected.

A further embodiment of the invention relates to a nucleic acid molecule for the detection of the presence or absence of bacteria belonging to a group of bacteria of the genus *Staphylococcus*, characterised in that it makes it possible by means of nucleic acid hybridisation and/or nucleic acid amplification methods under reaction conditions that are suitable or are known per se to distinguish between bacteria to be detected and bacteria that are not to be detected and that the distinction is possible or is facilitated by a differing nucleic acid sequence at at least one base position in

- (i) the region 54 to 83 of SEQ ID NO 1, or
- (ii) the region 100 to 166 of SEQ ID NO 1, or
- (iii) the sequence that is complementary to (i) or (ii)

in the genomic DNA and/or RNA of bacteria to be detected and bacteria that are not to be detected.

A further embodiment of the invention relates to a nucleic acid molecule of SEQ ID NO 1 or of its complementary sequence, especially for the detection according to the invention.

A further embodiment of the invention relates to a nucleic acid molecule having a sequence that is shorter than a nucleic acid molecule according to SEQ ID NO 1, namely

- (i) a sequence of the region or in the region of the nucleotide positions 54 to 83, or
- (ii) a sequence of the region or in the region of the nucleotide positions 100 to 166, or
- (iii) a sequence that is complementary to a sequence according to (i) or (ii).

A further embodiment of the invention relates to a nucleic acid molecule having a sequence that is shorter than a nucleic acid molecule according to SEQ ID NO 1, namely

- (i) SEQ ID NO 2, or
- (ii) SEQ ID NO 3, or
- (iii) SEQ ID NO 4, or
- (iv) the sequences complementary to (i), (ii) and (iii), respectively.

A further embodiment of the invention relates to a further or different nucleic acid molecule, characterised in that in respect of its sequence in at least 10 successive nucleotides of its nucleotide chain

- (i) it is identical to a nucleic acid molecule according to any one of the preceding claims, or
- (ii) it corresponds in 9 out of 10 successive nucleotides to a nucleic acid molecule according to any one of the preceding claims, or
- (iii) it corresponds in 8 out of 10 successive nucleotides to a nucleic acid molecule according to any one of the preceding claims, or
- (iv) it is at least 90% homologous to a nucleic acid molecule according to any one of the preceding claims.

Such a nucleic acid molecule may be characterised in that it is from 10 to 250, preferably from 15 to 30, nucleotides long.

An example of a nucleic acid molecule according to (i) is characterised by SEQ ID NO 5.

A nucleic acid molecule according to the invention may be characterised in that it is single-stranded or double-stranded.

A nucleic acid molecule according to the invention may be characterised in that it is present

- (i) as DNA, or
- (ii) as RNA corresponding to (i), or
- (iii) as PNA (see below),

the nucleic acid molecule, where appropriate, being modified in a manner known *per se* for analytical detection methods, especially methods based on hybridisation and/or amplification.

Such a nucleic acid molecule may be characterised in that the nucleic acid molecule is modified by the replacement of up to 10% of the nucleotides, especially 1 or 2 nucleotides, by analogous components known *per se* for probes and/or primers, especially by nucleotides that do not occur naturally in bacteria.

A nucleic acid molecule according to the invention may be characterised in that the nucleic acid molecule is modified or labelled or is additionally modified or labelled in that it comprises, in a manner known *per se* for analytical detection methods, one or more radioactive groups, coloured groups, fluorescent groups, groups for immobilisation on a solid phase and/or groups for an indirect or direct reaction, especially an enzymatic reaction, especially using antibodies, antigens, enzymes and/or substances having an affinity for enzymes or enzyme complexes.

A further embodiment of the invention relates to a kit for analytical detection methods, especially for the detection of bacteria of the genus *Staphylococcus*, characterised by one or more nucleic acid molecules according to the invention.

A further embodiment of the invention relates to a use of one or more nucleic acid molecules according to the invention or of a kit according to the invention for the detection of the presence or absence of bacteria belonging to a group of bacteria of the genus *Staphylococcus*.



That use may be characterised in that the group of bacteria of the genus *Staphylococcus* comprises various strains of *Staphylococcus aureus*.

That use may be characterised in that the group of bacteria of the genus *Staphylococcus* comprises exclusively *Staphylococcus aureus* strains.

Those uses may be characterised in that nucleic acid hybridisation and/or nucleic acid amplification is carried out.

That use may be characterised in that a polymerase chain reaction is carried out as nucleic acid amplification.

Those uses may be characterised in that the detection is carried out by distinguishing between the bacteria to be detected and bacteria that are not to be detected on the basis of differences in the genomic DNA and/or RNA at at least one nucleotide position in the region of a nucleic acid molecule according to the invention.

That use may be characterised in that the distinction is made on the basis of differences in the region of a nucleic acid molecule of SEQ ID NO 1.

To detect the group of microorganisms in question, nucleic acids, preferably genomic DNA, are first released from the cells contained in a sample or bacterial culture to be investigated. By means of nucleic acid hybridisation, the **direct** detection of organism-specific nucleic acid sequences in the sample to be investigated can then be

effected using the organism-specific oligonucleotides according to the invention as probe. Various methods known to the person skilled in the art are suitable for that purpose, such as, for example, "Southern blot" or "dot blot".

Preference is given, however, above all on account of the greater sensitivity, to an **indirect** detection method in which the DNA/RNA sequences sought are first amplified by means of the above-mentioned methods for the amplification of nucleic acids, preferably PCR.

The amplification of released DNA/RNA using the mentioned methods can be effected using organism-specific oligonucleotides as primers. In that case, specific amplification products are formed only when DNA/RNA of the microorganism to be detected is present. By a subsequent detection reaction using organism-specific oligonucleotides as probes, it is possible to increase the specificity of the detection method. For that subsequent detection reaction it is also possible to use non-organism-specific oligonucleotides as probes.

Alternatively, the nucleic acid amplification may be carried out in the presence of one or more non-specific oligonucleotides, so that possibly also DNA/RNA of other microorganisms that are not to be detected may be amplified. Such an amplification method is generally less specific and should therefore be backed up by a subsequent detection reaction using one or more organism-specific oligonucleotide(s) as probe(s).

Various methods are known to the person skilled in the art by means of which the amplification products that are formed in the indirect methods can be detected. These include, *inter alia*, visualisation by means of gel electrophoresis, the hybridisation of probes to immobilised reaction products [coupled to nylon or nitrocellulose filters ("Southern blots") or, for example, to beads or microtitre plates] and the hybridisation of the amplification products to immobilised probes (e.g. "reverse dot blots" or beads or microtitre plates coupled with probes).

A large number of different variants have been described by means of which organism-specific oligonucleotides (e.g. probes and primers) can be labelled or modified for the described direct or indirect detection methods. Thus, they may contain, for example, radioactive, coloured or fluorescent groups or groups that have been modified or that modify in some other manner, for example antibodies, antigens, enzymes or other substances having an affinity for enzymes or enzyme complexes. Probes and primers may be either naturally occurring or synthetically produced double-stranded or single-stranded DNA or RNA or modified forms of DNA or RNA, such as, for example, PNA (in those molecules, the sugar units are replaced by amino acids or peptides). Individual nucleotides or several nucleotides of the probes or primers may be replaced by analogous components (such as, for example, nucleotides that do not occur naturally in the target nucleic acid). In the above-mentioned indirect detection methods, detection may also be carried out using an internally labelled amplification product. This can be effected, for example, by the

integration of modified nucleoside triphosphates (e.g. coupled to digoxigenin or fluorescein) during the amplification reaction.

Suitable organism-specific oligonucleotides according to the invention are nucleic acids, preferably from 10 to 250 bases long and especially from 15 to 30 bases long, that correspond at least in a 10 base long sequence to the sequences 1 to 5 given below or to their complementary sequences. Relatively small differences (1 or 2 bases) in that 10 base long sequence are possible without loss of the requisite specificity in the amplification and/or hybridisation. The person skilled in the art will know that in the event of such relatively small differences the reaction conditions need to be altered accordingly.

The DNA sequence of *Staphylococcus aureus* (ATCC 6538) in the region of the 23S rDNA (1-113) and of the 23S/5S intergenic region (114-171) is:

(sequence 1 = SEQ ID NO 1)

```
TTTCCCAACTTCGGTTATAAGATCCCTCAAAGATGATGAGGTTAATAGGTTTCGAGGTGG
AAGCATGGTGACATGTGGAGCTGACGAATACTAATCGATCGAAGACTTAATCAAAATAA
ATGTTTTGCGAAGCAAAATCACTTTTACTTACTATCTAGTTTTGAATGTATAA
```

The sequence in the region of the 23S/5S intergenic region was determined for 8 *Staphylococcus aureus* strains and for at least one representative of each of the following species: *Staphylococcus auricularis*, *Staphylococcus capitis*, *Staphylococcus cohnii*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus hominis*, *Staphylococcus saccharolyticus*, *Staphylococcus*

*saprophyticus*, *Staphylococcus simulans*, *Staphylococcus warneri*, *Staphylococcus xylosus*. The sequence comparisons revealed that the sequences of the different species have a high degree of homology in that region. Three regions having high sequence variability can, however, be detected, namely from nucleotide positions 64 to 73, 110 to 121 and 127 to 156. Oligonucleotides suitable for the detection of bacteria of the genus *Staphylococcus* can therefore be derived from the nucleotide sequences of the different *Staphylococcus* species in the regions 54 to 83 and 100 to 166. For example, from sequence 1 a number of oligonucleotides can be derived that span either fully or partially the variable nucleotide positions 64 to 73, 110 to 121 and 127 to 156 and that are suitable as primers or probes for the selective detection of bacteria of the species *Staphylococcus aureus*.

From sequence 1 there were derived the following oligonucleotides that are especially suitable as primers for the PCR (sequence 2 and sequence 3) and as probes (sequence 4 and sequence 5):

Oligonucleotide Sa1:	(sequence 2 = SEQ ID NO 2)
	5'-GTGGAAGCATGGTGACAT-3'
Oligonucleotide Sa2:	(sequence 3 = SEQ ID NO 3)
	5'-TAAGTAAAAGTGATTTTGCT TCG-3'
Oligonucleotide Sa3:	(sequence 4 = SEQ ID NO 4)
	5'-CATTTATTTTGATTAAGTCT-3'
Oligonucleotide Sa4:	(sequence 5 = SEQ ID NO 5)
	5'-CATTTAAATTTGATTAAGTC-3'

**Example 1:** Detection of bacteria of the species  
*Staphylococcus aureus* by polymerase chain reaction

DNA was isolated from pure cultures of the bacteria listed in Table 1 by means of standard procedures. Approximately from 10 to 100 ng of the DNA preparations were then used in the PCR in the presence of 0.6  $\mu$ M oligonucleotide Sa1 (SEQ ID NO 2) and 0.6  $\mu$ M oligonucleotide Sa2 (SEQ ID NO 3), 200  $\mu$ M dNTP's (N = A/C/G/T mixture; Boehringer Mannheim), 2 mM  $MgCl_2$ , 16 mM  $(NH_4)_2SO_4$ , 67 mM Tris/HCl (pH 8.8), 0.01% Tween 20 and 0.03 U/ $\mu$ l *Taq*-polymerase (Biomaster). The PCR was carried out in a Perkin-Elmer 9600 thermocycler having the following thermoprofile:

- initial denaturing	94°C	3 min
- amplification (35 cycles)	92°C	30 sec
	60°C	10 sec
- final synthesis	72°C	5 min.

After completion of the PCR reaction, the amplification products were separated by means of agarose gel electrophoresis and visualised by staining with ethidium bromide. The expected product of 94 bp in length was observed only in the cases in which DNA of strains of the species *Staphylococcus aureus* was present (compare **Table 1**), but not in the presence of DNA of the other tested bacteria. After the end of the run, the DNA contained in the gels was transferred to nylon filters by means of standard methods and was hybridised with an equimolar mixture of the oligonucleotides Sa3 (SEQ ID NO 4) and Sa4 (SEQ ID NO 5)

biotinylated at the 5'-end in order to investigate the specificity. Hybridisation was effected in 5 x SSC, 2% blocking reagent, 0.1% lauryl sarcosine, 0.02% SDS and 20 pmol/ml of probe for 4 hours at 43°C. Washing was effected in 2 x SSC, 0.1% SDS for 1 x 5 min at room temperature and for 1 x 5 min at 43°C. Detection was effected according to standard methods by means of alkaline phosphatase conjugates (extravidin, SIGMA, # E-2636) in the presence of 5-bromo-4-chloro-3-indolyl phosphate and 4-nitro-blue tetrazolium chloride (Boehringer Mannheim).

A band was observed on the filters only in the cases in which a band had previously been visible on the agarose gel (see **Table 1**). Thus, the presence of all the fifty tested *Staphylococcus aureus* strains was detected by means of PCR and by means of hybridisation. Of the total of fifty *Staphylococcus aureus* strains tested, two are coagulase-negative (BioteCon 7030 and BioteCon 7044), which are also detected by the PCR method. On the other hand, none of the tested bacterial strains not belonging to that species was detected using that system.

**Table 1:** Results of the PCR amplification using oligonucleotides Sa1 and Sa2 (SEQ ID NO 2 and SEQ ID NO 3) and subsequent hybridisation using oligonucleotides Sa3/Sa4 (SEQ ID NO 4 and SEQ ID NO 5).

Species	Designation of strain	PCR	Hybridisation
<i>Staphylococcus aureus</i>	ATCC 6538	+	+
<i>Staphylococcus aureus</i>	BioteCon 194	+	+
<i>Staphylococcus aureus</i>	BioteCon 195	+	+
<i>Staphylococcus aureus</i>	BioteCon 196	+	+
<i>Staphylococcus aureus</i>	BioteCon 197	+	+
<i>Staphylococcus aureus</i>	BioteCon 494	+	+
<i>Staphylococcus aureus</i>	BioteCon 514	+	+
<i>Staphylococcus aureus</i>	BioteCon 528	+	+
<i>Staphylococcus aureus</i>	BioteCon 529	+	+
<i>Staphylococcus aureus</i>	BioteCon 530	+	+
<i>Staphylococcus aureus</i>	BioteCon 531	+	+
<i>Staphylococcus aureus</i>	BioteCon 532	+	+
<i>Staphylococcus aureus</i>	BioteCon 533	+	+
<i>Staphylococcus aureus</i>	BioteCon 534	+	+
<i>Staphylococcus aureus</i>	BioteCon 535	+	+
<i>Staphylococcus aureus</i>	BioteCon 4286	+	+
<i>Staphylococcus aureus</i>	BioteCon 4287	+	+
<i>Staphylococcus aureus</i>	BioteCon 4288	+	+
<i>Staphylococcus aureus</i>	BioteCon 4289	+	+
<i>Staphylococcus aureus</i>	ATCC 25923	+	+
<i>Staphylococcus aureus</i>	BioteCon 7010	+	+
<i>Staphylococcus aureus</i>	BioteCon 7011	+	+
<i>Staphylococcus aureus</i>	BioteCon 7012	+	+
<i>Staphylococcus aureus</i>	BioteCon 7013	+	+
<i>Staphylococcus aureus</i>	BioteCon 7014	+	+
<i>Staphylococcus aureus</i>	BioteCon 7015	+	+
<i>Staphylococcus aureus</i>	BioteCon 7016	+	+
<i>Staphylococcus aureus</i>	BioteCon 7017	+	+
<i>Staphylococcus aureus</i>	BioteCon 7018	+	+
<i>Staphylococcus aureus</i>	BioteCon 7019	+	+
<i>Staphylococcus aureus</i>	BioteCon 7020	+	+
<i>Staphylococcus aureus</i>	BioteCon 7021	+	+
<i>Staphylococcus aureus</i>	BioteCon 7022	+	+
<i>Staphylococcus aureus</i>	BioteCon 7024	+	+
<i>Staphylococcus aureus</i>	BioteCon 7025	+	+
<i>Staphylococcus aureus</i>	BioteCon 7027	+	+
<i>Staphylococcus aureus</i>	BioteCon 7028	+	+
<i>Staphylococcus aureus</i>	BioteCon 7029	+	+
<i>Staphylococcus aureus</i>	BioteCon 7030	+	+
<i>Staphylococcus aureus</i>	BioteCon 7031	+	+
<i>Staphylococcus aureus</i>	BioteCon 7032	+	+
<i>Staphylococcus aureus</i>	BioteCon 7034	+	+



Species	Designation of strain	PCR	Hybridisation
<i>Staphylococcus aureus</i>	BioteCon 7035	+	+
<i>Staphylococcus aureus</i>	BioteCon 7036	+	+
<i>Staphylococcus aureus</i>	BioteCon 7039	+	+
<i>Staphylococcus aureus</i>	BioteCon 7040	+	+
<i>Staphylococcus aureus</i>	BioteCon 7041	+	+
<i>Staphylococcus aureus</i>	BioteCon 7042	+	+
<i>Staphylococcus aureus</i>	BioteCon 7043	+	+
<i>Staphylococcus aureus</i>	BioteCon 7044	+	+
<i>Staphylococcus arlettae</i>	DSM 20672	-	-
<i>Staphylococcus auricularis</i>	DSM 20609	-	-
<i>Staphylococcus capitis</i> subsp. <i>ureolyticus</i>	BioteCon 2687	-	-
<i>Staphylococcus caprae</i>	DSM 20608	-	-
<i>Staphylococcus carnosus</i>	DSM 20501	-	-
<i>Staphylococcus caseolyticus</i>	DSM 20597	-	-
<i>Staphylococcus chromogenes</i>	DSM 20454	-	-
<i>Staphylococcus cohnii</i> subsp. <i>cohnii</i> ,	DSM 20260	-	-
<i>Staphylococcus delphini</i>	DSM 20771	-	-
<i>Staphylococcus epidermidis</i>	BioteCon 515	-	-
<i>Staphylococcus equorum</i>	DSM 20674	-	-
<i>Staphylococcus gallinarum</i>	DSM 20610	-	-
<i>Staphylococcus haemolyticus</i>	BioteCon 2847	-	-
<i>Staphylococcus hominis</i>	DSM 20328	-	-
<i>Staphylococcus hyicus</i>	DSM 20459	-	-
<i>Staphylococcus intermedius</i>	DSM 20373	-	-
<i>Staphylococcus kloosii</i>	DSM 20676	-	-
<i>Staphylococcus lentus</i>	DSM 6672	-	-
<i>Staphylococcus lugdunensis</i>	BioteCon 2681	-	-
<i>Staphylococcus muscae</i>	DSM 7068	-	-
<i>Staphylococcus</i> <i>saccharolyticus</i>	DSM 20359	-	-
<i>Staphylococcus saprophyticus</i>	BioteCon 2685	-	-
<i>Staphylococcus schleiferi</i> subsp. <i>schleiferi</i>	DSM 4808	-	-
<i>Staphylococcus sciuri</i>	DSM 6671	-	-
<i>Staphylococcus simulans</i>	DSM 20322	-	-
<i>Staphylococcus warneri</i>	DSM 20036	-	-
<i>Staphylococcus xylosus</i>	BioteCon 2683	-	-
<i>Bacillus cereus</i>	DSM 31	-	n.p.
<i>Bacillus coagulans</i>	DSM 1	-	n.p.
<i>Bacillus brevis</i>	DSM 30	-	n.p.
<i>Bacillus megaterium</i>	DSM 32	-	n.p.
<i>Bacillus thuringiensis</i>	DSM 350	-	n.p.
<i>Bacillus badius</i>	DSM 23	-	n.p.
<i>Bacillus sphaericus</i>	BioteCon 3136	-	n.p.
<i>Bacillus subtilis</i>	ATCC 6633	-	n.p.
<i>Bacillus circulans</i>	BioteCon 4926	-	n.p.
<i>Bacillus polymyxa</i>	ATCC 8523	-	n.p.
<i>Bacillus mycoides</i>	BioteCon 4928	-	n.p.
<i>Bacillus stearothermophilus</i>	DSM 456	-	n.p.
<i>Pseudomonas aeruginosa</i>	BiotCon 682	-	n.p.

Species	Designation of strain	PCR	Hybridisation
<i>Pseudomonas fluorescens</i>	Biotecon 2439	-	n.p.
<i>Pseudomonas cepacia</i>	BioteCon 672	-	n.p.
<i>Pseudomonas chlororaphis</i>	BioteCon 1753	-	n.p.
<i>Pseudomonas citronellolis</i>	DSM 50332	-	n.p.
<i>Pseudomonas mendocina</i>	DSM 50017	-	n.p.
<i>Pseudomonas pickettii</i>	BioteCon 3323	-	n.p.
<i>Pseudomonas fragi</i>	DSM 3456	-	n.p.
<i>Pseudomonas hydrophila</i>	BioteCon 4883	-	n.p.
<i>Pseudomonas putida</i>	BioteCon 4884	-	n.p.
<i>Pseudomonas oleovorans</i>	DSM 1045	-	n.p.
<i>Pseudomonas pseudoalcaligenes</i>	DSM 50188	-	n.p.
<i>Pseudomonas syringae</i>	DSM 10604	-	n.p.
<i>Pseudomonas mendocina</i>	DSM 50017	-	n.p.
<i>Pseudomonas corrugata</i>	DSM 7228	-	n.p.
<i>Lactobacillus brevis</i>	DSM 1267	-	n.p.
<i>Lactobacillus viridescens</i>	BioteCon 2592	-	n.p.
<i>Lactobacillus kefiranofaciens</i>	DSM 5016	-	n.p.
<i>Lactobacillus lindneri</i>	BioteCon 2599	-	n.p.
<i>Lactobacillus fructivorans</i>	BioteCon 2598	-	n.p.
<i>Lactobacillus casei</i>	DSM 20011	-	n.p.
<i>Lactobacillus vaginalis</i>	DSM 5837	-	n.p.
<i>Lactobacillus fermentum</i>	BioteCon 2597	-	n.p.
<i>Lactobacillus intestinalis</i>	DSM 6629	-	n.p.
<i>Lactobacillus bifermentans</i>	DSM 20003	-	n.p.
<i>Lactobacillus curvatus</i>	DSM 20019	-	n.p.
<i>Lactobacillus coryniformis</i>	DSM 20004	-	n.p.
<i>subsp. torquens</i>			
<i>Lactobacillus acidophilus</i>	BioteCon 786	-	n.p.
<i>Lactobacillus alimentarius</i>	DSM 20249	-	n.p.
<i>Lactobacillus fructosus</i>	DSM 20349	-	n.p.
<i>Lactobacillus malefermentans</i>	DSM 20177	-	n.p.
<i>Lactobacillus kefir</i>	DSM 20485	-	n.p.
<i>Lactobacillus salivarius</i>	DSM 20492	-	n.p.
<i>subsp. salivarius</i>			
<i>Lactobacillus homohiochii</i>	DSM 20571	-	n.p.
<i>Lactobacillus sanfrancisco</i>	DSM 20663	-	n.p.
<i>Lactobacillus aviarius</i>	DSM 20655	-	n.p.
<i>Lactobacillus ruminis</i>	DSM 20403	-	n.p.
<i>Leuconostoc mesenteriodes</i>	DSM 20240	-	n.p.
<i>subsp. mesenteriodes</i>			
<i>Leuconostoc mesenteriodes</i>	DSM 20071	-	n.p.
<i>subsp. dextranicum</i>			
<i>Leuconostoc mesenteriodes</i>	DSM 20346	-	n.p.
<i>subsp. cremoris</i>			
<i>Leuconostoc lactis</i>	DSM 20202	-	n.p.
<i>Leuconostoc oenos</i>	DSM 20255	-	n.p.
<i>Leuconostoc gelidum</i>	DSM 5578	-	n.p.
<i>Leuconostoc carnosum</i>	DSM 5576	-	n.p.
<i>Leuconostoc citreum</i>	DSM 20188	-	n.p.
<i>Leuconostoc paramesenteroides</i>	DSM 20288	-	n.p.

Species	Designation of strain	PCR	Hybridisation
<i>Leuconostoc fallax</i>	DSM 20189	-	n.p.
<i>Leuconostoc pseudomesenteroides</i>	DSM 5625	-	n.p.
<i>Streptococcus downei</i>	DSM 5635	-	n.p.
<i>Streptococcus</i> sp.	DSM 6176	-	n.p.
<i>Streptococcus parauberis</i>	DSM 6631	-	n.p.
<i>Streptococcus gordonii</i>	DSM 6777	-	n.p.
<i>Streptococcus</i> sp.	DSM 20061	-	n.p.
<i>Streptococcus equinus</i>	DSM 20062	-	n.p.
<i>Streptococcus salivarius</i> subsp. <i>thermophilus</i>	DSM 20259	-	n.p.
<i>Streptococcus canis</i>	DSM 20386	-	n.p.
<i>Streptococcus cricetus</i>	DSM 20562	-	n.p.
<i>Streptococcus anginosus</i>	DSM 20563	-	n.p.
<i>Streptococcus rattus</i>	DSM 20564	-	n.p.
<i>Streptococcus pneumoniae</i>	DSM 20566	-	n.p.
<i>Streptococcus pleomorphus</i>	DSM 20574	-	n.p.
<i>Streptococcus iniae</i>	DSM 20576	-	n.p.
<i>Streptococcus hansenii</i>	DSM 20583	-	n.p.
<i>Streptococcus ferus</i>	DSM 20646	-	n.p.
<i>Streptococcus alactolyticus</i>	DSM 20728	-	n.p.
<i>Streptococcus hyointestinalis</i>	DSM 20770	-	n.p.
<i>Pediococcus parvulus</i>	DSM 20332	-	n.p.
<i>Campylobacter jejuni</i> subsp. <i>jejuni</i>	DSM 4688	-	n.p.
<i>Citrobacter freundii</i>	DSM 30040	-	n.p.
<i>Shigella flexneri</i>	DSM 4782	-	n.p.
<i>Pectinatus cerevisiiphilus</i>	DSM 2834	-	n.p.
<i>Pediococcus inopinatus</i>	DSM 20285	-	n.p.
<i>Proteus mirabilis</i>	BioteCon 4701	-	n.p.
<i>Megasphaera cerevisiae</i>	DSM 20461	-	n.p.
<i>Escherichia coli</i>	BioteCon 4949	-	n.p.

n.p.: The hybridisation was not performed.

Our ref.: 9366

International Patent Application PCT/EP 98/04510

BioteCon GmbH

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Patent claims 1 to 33  
according to Art. 34 Chapter II PCT

1. Kit for the analytical detection of bacteria of the genus *Staphylococcus*, **characterised** by more than one nucleic acid molecule as primer and/or probe, wherein at least one of the nucleic acid molecules hybridises selectively to RNA or DNA of a group of bacteria of the genus *Staphylococcus*, wherein it contains at least 10 successive nucleotides of the region from -113 to +58 relative to the 3'-end of the 23S rDNA of a *Staphylococcus* isolate or their complementary nucleotides.
2. Kit for the analytical detection of bacteria of the genus *Staphylococcus*, **characterised** by more than one nucleic acid molecule as primer and/or probe, wherein at least one of the nucleic acid molecules hybridises selectively to RNA or DNA of a group of bacteria of the genus *Staphylococcus*, wherein it contains at least 10 successive nucleotides of the region from -113 to +58 relative to the 3'-end of the 23S rDNA of *Staphylococcus aureus* (ATCC 6538) or their complementary nucleotides.
3. Kit for the analytical detection of bacteria of the genus *Staphylococcus*, **characterised** by more than one

nucleic acid molecule as primer and/or probe, wherein at least one of the nucleic acid molecules hybridises selectively to RNA or DNA of a group of bacteria of the genus *Staphylococcus*, wherein it contains at least 10 successive nucleotides of the region from

- (i) nucleotide position 54 to 83 of SEQ ID NO 1, or
- (ii) nucleotide position 100 to 166 of SEQ ID NO 1, or
- (iii) the sequences complementary to (i) or (ii).

4. Kit for the analytical detection of bacteria of the genus *Staphylococcus*, **characterised** by more than one nucleic acid molecule as primer and/or probe for the detection of the presence or absence of bacteria belonging to a group of bacteria of the genus *Staphylococcus*, wherein at least one of the nucleic acid molecules makes it possible by means of nucleic acid amplification and/or nucleic acid hybridisation methods under suitable reaction conditions to distinguish between bacteria to be detected and bacteria that are not to be detected, and wherein the distinction is possible by virtue of a differing nucleic acid sequence at at least one base position in the region of SEQ ID NO: 1, or of its complementary sequence, in the genomic DNA and/or RNA of bacteria to be detected and bacteria that are not to be detected .

5. Kit for the analytical detection of bacteria of the genus *Staphylococcus*, **characterised** by more than one nucleic acid molecule as primer and/or probe for the detection of the presence or absence of bacteria belonging to a group of bacteria of the genus *Staphylococcus*, wherein at least one of the nucleic acid molecules makes it possible by means of nucleic acid hybridisation and/or

nucleic acid amplification methods under reaction conditions known per se to distinguish between bacteria to be detected and bacteria that are not to be detected, and wherein the distinction is possible by virtue of a differing nucleic acid sequence at at least one base position in

- (i) the region 54 to 83 of SEQ ID NO 1, or
- (ii) the region 100 to 166 of SEQ ID NO 1, or
- (iii) the sequence that is complementary to the region according to (i) or (ii)

in the genomic DNA and/or RNA of bacteria to be detected and bacteria that are not to be detected.

6. Kit according to claim 5, **characterised** by a nucleic acid molecule that has the SEQ ID NO 1 or its complementary sequence.

7. Kit according to claim 6, **characterised** by a nucleic acid molecule having a sequence that is shorter than a nucleic acid molecule according to claim 6, namely

- (i) a sequence of the region or in the region of the nucleotide positions 54 to 83, or
- (ii) a sequence of the region or in the region of the nucleotide positions 100 to 166, or
- (iii) a sequence that is complementary to a sequence according to (i) or (ii).

8. Kit according to claim 6, **characterised** by a nucleic acid molecule having a sequence that is shorter than a nucleic acid molecule according to claim 6, namely

- (i) SEQ ID NO 2, or
- (ii) SEQ ID NO 3, or

- (iii) SEQ ID NO 4, or
- (iv) the sequences complementary to (i), (ii) and (iii), respectively.

9. Kit according to any one of the preceding claims, **characterised** by a nucleic acid molecule that differs from a nucleic acid molecule according to any one of the preceding claims but that in respect of its sequence in at least 10 successive nucleotides of its nucleotide chain

- (i) is identical to the nucleic acid molecule according to any one of the preceding claims, or
- (ii) corresponds in 9 out of 10 successive nucleotides to the nucleic acid molecule according to any one of the preceding claims, or
- (iii) corresponds in 8 out of 10 successive nucleotides to the nucleic acid molecule according to any one of the preceding claims, or
- (iv) is at least 90% homologous to the nucleic acid molecule according to any one of the preceding claims.

10. Kit according to claim 9, **characterised** in that the nucleic acid molecule is from 10 to 250, preferably from 15 to 30, nucleotides long, especially characterised in that it is the nucleic acid molecule having the sequence SEQ ID NO 5.

11. Kit according to any one of the preceding claims, **characterised** in that the nucleic acid molecule is single-stranded or double-stranded.

12. Kit according to any one of the preceding claims, **characterised** in that the nucleic acid molecule is present

(i) as DNA, or  
(ii) as RNA corresponding to (i), or  
(iii) as PNA,  
the nucleic acid molecule, where appropriate, being  
modified in a manner known *per se* for analytical detection  
methods, especially methods based on hybridisation and/or  
amplification.

13. Kit according to claim 12, **characterised** in that the  
nucleic acid molecule is modified by the replacement of up  
to 10% of the nucleotides, especially 1 or 2 nucleotides,  
by analogous components known *per se* for probes and/or  
primers, especially by nucleotides that do not occur  
naturally in bacteria.

14. Kit according to claim 12 or claim 13, **characterised**  
in that the nucleic acid molecule is modified or labelled  
or is additionally modified or labelled in that it  
comprises, in a manner known *per se* for analytical  
detection methods, one or more radioactive groups, coloured  
groups, fluorescent groups, groups for immobilisation on a  
solid phase and/or groups for an indirect or direct  
reaction, especially an enzymatic reaction, especially  
using antibodies, antigens, enzymes and/or substances  
having an affinity for enzymes or enzyme complexes, or it  
comprises, in a manner known *per se* for analytical  
detection methods, groups that have been modified or that  
modify in some other manner.

15. Use of a kit according to any one of the preceding  
claims for the detection of the presence or absence of



bacteria belonging to a group of bacteria of the genus *Staphylococcus*.

16. Use according to claim 15, **characterised** in that the group of bacteria of the genus *Staphylococcus* comprises various strains of *Staphylococcus aureus*.

17. Use according to claim 16, **characterised** in that the group of bacteria of the genus *Staphylococcus* comprises exclusively *Staphylococcus aureus* strains.

18. Use according to any one of claims 15 to 17, **characterised** in that nucleic acid hybridisation and/or nucleic acid amplification is carried out.

19. Use according to claim 18, **characterised** in that a polymerase chain reaction is carried out as nucleic acid amplification.

20. Use according to any one of claims 15 to 19, **characterised** in that the detection is carried out by distinguishing between the bacteria to be detected and bacteria that are not to be detected on the basis of differences in the genomic DNA and/or RNA at at least one nucleotide position in the region of a nucleic acid molecule according to any one of claims 1 to 14.

21. Use according to claim 20, **characterised** in that the distinction is made on the basis of differences in the region of a nucleic acid molecule according to claim 6. ✓

22. Nucleic acid molecule that hybridises selectively to RNA or DNA of a group of bacteria of the genus *Staphylococcus*, **characterised** in that it contains at least 10 successive nucleotides of the region from -113 to +58 relative to the 3'-end of the 23S rDNA of a *Staphylococcus* isolate or their complementary nucleotides, excluding a nucleic acid molecule that has a sequence according to Figures 1 to 10.

23. Nucleic acid molecule that hybridises selectively to RNA or DNA of a group of bacteria of the genus *Staphylococcus*, **characterised** in that it contains at least 10 successive nucleotides of the region from -113 to +58 relative to the 3'-end of the 23S rDNA of *Staphylococcus aureus* (ATCC 6538) or their complementary nucleotides, excluding a nucleic acid molecule that has a sequence according to Figures 1 to 10.

24. Nucleic acid molecule that hybridises selectively to RNA or DNA of a group of bacteria of the genus *Staphylococcus*, **characterised** in that it contains at least 10 successive nucleotides of the region from  
(i) nucleotide position 54 to 83 of SEQ ID NO 1, or  
(ii) nucleotide position 100 to 166 of SEQ ID NO 1, or  
(iii) the sequences complementary to (i) or (ii),  
excluding a nucleic acid molecule that has a sequence according to Figures 1 to 10.

25. Nucleic acid molecule for the detection of the presence or absence of bacteria belonging to a group of bacteria of the genus *Staphylococcus*, **characterised** in that it makes it possible by means of nucleic acid hybridisation

and/or nucleic acid amplification methods under suitable reaction conditions to distinguish between bacteria to be detected and bacteria that are not to be detected and that the distinction is possible by virtue of a differing nucleic acid sequence at at least one base position in the region of SEQ ID NO: 1, or of its complementary sequence, in the genomic DNA and/or RNA of bacteria to be detected and bacteria that are not to be detected, excluding a nucleic acid molecule that has a sequence according to Figures 1 to 10.

26. Nucleic acid molecule for the detection of the presence or absence of bacteria belonging to a group of bacteria of the genus *Staphylococcus*, **characterised** in that it makes it possible by means of nucleic acid hybridisation and/or nucleic acid amplification methods under reaction conditions known *per se* to distinguish between bacteria to be detected and bacteria that are not to be detected and that the distinction is possible by virtue of a differing nucleic acid sequence at at least one base position in

- (i) the region 54 to 83 of SEQ ID NO 1, or
- (ii) the region 100 to 166 of SEQ ID NO 1, or
- (iii) the sequence that is complementary to (i) or (ii),

in the genomic DNA and/or RNA of bacteria to be detected and bacteria that are not to be detected, excluding a nucleic acid molecule that has a sequence according to Figures 1 to 10.

27. Nucleic acid molecule, **characterised** in that it has the SEQ ID NO 1 or its complementary sequence.

28. Nucleic acid molecule having a sequence that is shorter than a nucleic acid molecule according to claim 27, namely

- (i) a sequence of the region or in the region of the nucleotide positions 54 to 83, or
- (ii) a sequence of the region or in the region of the nucleotide positions 100 to 166, or
- (iii) a sequence that is complementary to a sequence according to (i) or (ii).

29. Nucleic acid molecule having a sequence that is shorter than a nucleic acid molecule according to claim 27, namely

- (i) SEQ ID NO 3, or
- (ii) SEQ ID NO 4, or
- (iii) the sequences complementary to (i) and (ii), respectively.

30. Nucleic acid molecule, **characterised** in that in respect of its sequence in at least 10 successive nucleotides of its nucleotide chain

- (i) it is identical to a nucleic acid molecule according to any one of claims 22 to 29, or
- (ii) it corresponds in 9 out of 10 successive nucleotides to a nucleic acid molecule according to any one of claims 22 to 29, or
- (iii) it corresponds in 8 out of 10 successive nucleotides to a nucleic acid molecule according to any one of claims 22 to 29, or
- (iv) it is at least 90% homologous to a nucleic acid molecule according to any one of claims 22 to 29.

31. Nucleic acid molecule, **characterised** in that it has the SEQ ID NO 5 or its complementary sequence.

32. Nucleic acid molecule according to any one of claims 22 to 30, **characterised** in that it is from 10 to 250, preferably from 15 to 30, nucleotides long.

33. Nucleic acid molecule according to any one of claims 22 to 32, **characterised** in that the nucleic acid molecule is single-stranded or double-stranded.

**Abstract**

The present invention relates to a nucleic acid molecule or molecules and to a method for the detection of bacteria of the genus *Staphylococcus*, especially *Staphylococcus aureus*. The invention relates also to a test kit or test kits for carrying out the mentioned detection methods.

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ART 34.11.1.

Fig. 1

TAGTCACCAG ACATATGAAT GTAATTTATA CATTCAAAAC TAGATAGTAA  
GTAAAAGTGA 60

TTTTGCTTCG CAAAACATTT ATTTTGATTA AGTCTTCGAT CGATTAGTAT  
TCGTCAGCTC 120

CACATGTCAC CATGCTTCCA CCTCGAACCT ATTAACCTCA TCATCTTTGA  
GGGATCTTAT 180

AACCGAAGTT GGGAAATCTC ATCTTGAGGG GGGCTTCATG CTTAGATGCT  
TTCAGCACTT 240

ATCCCGTCCA CACATAGCTA CCCAGCTATG CCGTTGGCAC GACAACTGGT  
ACACCAGAGG 300

TATGTCCATC CCGGTCCTCT CGTACTAAGG ACAGCTCCTN TCAAATTTCC  
TACGNCCANG 360

ACGGATAGGG ACCGAACTGT TTTCACGACG GTNCTGAACC

400

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ART 34 ANDT

Fig. 2

TCGACTACCA TCGACGCTAA GGAGCTTAAC TTCTGTGTTC GGCATGGGAA  
CAGTGTGACT 60

CCTTGCTATA GTCACCAGAC ATATGAATGT AATTATACAT TCCAAACTAG  
ATAGTAAGTA 120

AAAGTGATTT GCTTCGCCAA ACATTTATTT TGATTAAGTC TTCGATCGAT  
TAGTATTCGT 180

CAGCTCCACA TGTCACCATG CTCCANCTN GAA

213



412

ATTCGTCAGC TCCACATGTC ACCATGCTTC CACCTCGAAC CTATTAACCT  
CATCATCTTT 60

GAGGGATCTT ATAACCGAAG TTGGGAAATC TCATCTTGAG GGGGGTTCAT  
GCTAGATGCT 120

TCAGACTATC CCGTCCACAC ATGTAACCAG NATGCGTGGA CGCATGGAAC  
AGGGATGTCA 180

TCCG

184

418

ART 34 AMBT

Fig. 4

CCCGTGAAAG ATGATGAGGT TAATAGGTTT GAGGTGGAAG CATGGTGACA  
TGTGGAGCGT 60

GACGAATACG TAATTGA

77

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ART 34 AMDT

Fig. 5

CGAATACTAA TCGATCGAAG ACTTAATCAA AATAAATGTT TTGCGACNAA

50

ART 34 AMCT

Fig. 6

AATCGTCGAA ACTTAATCAA AATAAATGTT TTGCGACAAA TCACTTTTAC  
TTACTATCTA 60



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## ART 34 AMDT

Fig. 9

```

                                     10 20 30
                                     TTTCCCAACTTCGGTTATAAGATCCCTCAA
                                     |||||
TCTAAGCATGAAGCCCCCTCAAGATGAGATTTCCCAACTTCGGTTATAAGATCCCTCAA
      2790      2800      2810      2820      2830      2840

      40      50      60      70      80      90
AGATGATGAGGTTAATAGGTTTCGAGGTGGAAGCATGGTGACATGTGGAGCTGACGAATAC
|||||
AGATGATGAGGTTAATAGGTTTCGAGGTGGAAGCATGGTGACATGTGGAGCTGACGAATAC
      2850      2860      2870      2880      2890      2900

      100      110      120      130      140      150
TAATCGATCGAAGACTTAATCAAAATAAATGTTTTCGCAAGCAAATCACTTTTACTTAC
|||||

TAATCGATCGAAGACTTAATCAA
      2910      2920
```

Fig. 10

```

                                     10 20 30
                                     TTTCCCAACTTCGGTTATAAGATCCCTCAA
                                     |||||
TCTAAGCATGAAGCCCCCTCAAGATGAGATTTCCCAACTTCGGTTATAAGATCCCTCAA
      7990      8000      8010      8020      8030      8040

      40      50      60      70      80      90
AGATGATGAGGTTAATAGGTTTCGAGGTGGAAGCATGGTGACATGTGGAGCTGACGAATAC
|||||
AGATGATGAGGTTAATAGGTTTCGAGGTGGAAGCATGGTGACATGTGGAGCTGACGAATAC
      8050      8060      8070      8080      8090      8100

      100      110      120      130      140      150
TAATCGATCGAAGACTTAATCAAAATAAATGTTTTCGCAAGCAAATCACTTTTACTTAC
|||||
TAATCGATCGAGGGCTTAACCAAAATAAATGTTTTCGCAAGCAAATCACTTTTACTTAC

      8110      8120      8130      8140      8150      8160

      160      170
TATCTAGTTTTGAATGTATAA
|||||
TATCTAGTTTTGAATGTATAAATTACATTCATATGTCTGGTGACTATAGCAAGGAGGTCA
      8170      8180      8190      8200      8210      8220
```

Type a plus sign (+) inside this box ☒

0010/PTO  
Rev. 04/93

U.S. Department of Commerce  
Patent and Trademark Office

# DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION

☐ Declaration Submitted with Initial Filing OR ☒ Declaration Submitted after Initial Filing

Attorney Docket Number 2727-99J

First Named Inventor Kornelia Berghof

## COMPLETE IF KNOWN

Application Number 09/463,209

Filing Date

Group Art Unit

Examiner Name

As a below named inventor, I hereby declare that

my residence, last office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

"Nucleic Acid Molecule, Test Kit and Use"

(Title of the Invention)

The specification of which

☐ is attached hereto

OR

☒ was filed on (MM/DD/YYYY)

07/21/1998

as United States Application Number or PCT International

(USSN 09/463,209)

Application Number

PCT/EP98/04510

and was amended on (MM/DD/YYYY)

(if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37 Code of Federal Regulations, §1.56

I hereby claim foreign priority benefits under Title 35, United States Code §115 (a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate, or §365 (d) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking one box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached?	
				YES	NO
197 31 292.6	Germany	07/21/1997	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
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			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

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I hereby claim the benefits under Title 35, United States Code §115(a) of any United States provisional application(s) listed below

Application Number(s)	Filing Date (MM/DD/YYYY)	Additional provisional application numbers are listed on a supplemental priority sheet attached hereto.
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## DECLARATION

Page 2

I hereby claim the benefit under Title 35, United States Code §120 of any United States application(s), or §363(c) of any PCT international application designating the United States of America, filed before and under the subject matter of each of the claims of this application is not included in the prior United States or PCT international application or the matter provided by the first paragraph of Title 35, United States Code §112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations §1.56 which becomes available between the filing date of the prior application and the receipt of PCT international filing date of this application.

U.S. Parent Application Number	PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number (if applicable)

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As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to conduct all business in the Patent and Trademark Office connected therewith:

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☐ List attorney(s) and/or agent(s) name and registration number below

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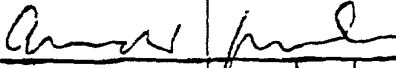
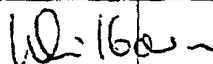
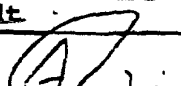
(Declaration for Utility or Design Patent Application (PTO/SB/01) (1-10.1)—page 2 of 4)



DECLARATION				ADDITIONAL INVENTOR(S) Supplemental Sheet			
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4 4  
3 3

Declaration for Utility or Design Patent Application (PTO/38/01) (1-10.1) - page - 4 -

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Inventor's Signature					Date	<u>21 03 00</u>	
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